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(54) **Title:** STRUCTURAL AND FUNCTIONAL CONSERVATION OF THE *C. ELEGANS* CLOCK GENE *clk–1*

(57) **Abstract**

The present invention relates to a *clk–1* gene which has a function at the level of cellular physiology involved in developmental rate and longevity, wherein *clk–1* mutants have a longer life and a altered cellular metabolism relative to the wild–type. There is also provided a method for the diagnosis and/or prognosis of cancer in a patient, which comprises the steps of: a) obtaining a tissue sample from the patient; b) analyzing DNA of the obtained tissue sample of step a) to determine if the human *clk–1* gene is altered, wherein alteration of the human *clk–1* gene is indicative of cancer. There is also provided a method of treatment of pathological conditions causing slowdown of physiological rate of tissue and/or organ in a patient.

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STRUCTURAL AND FUNCTIONAL CONSERVATION OF THE *C. ELEGANS* CLOCK GENE *clk-1*

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The invention relates to the identification of *clk-1* and to show that the *clk-1* gene complements the *clk-1* phenotype and restores normal longevity.

(b) Description of Prior Art

The activity of the gene *clk-1* in the nematode *Caenorhabditis elegans* controls how fast the worms live and how soon they die. In *clk-1* mutants, the timing of a wide range of physiological processes is deregulated. This leads to an average lengthening of such diverse processes as the worms' early cell cycles, their embryonic and post-embryonic development, and the period of rhythmic adult behaviors, such as swimming, pharyngeal pumping, and defecation (A. Wong et al., *Genetics* **139**, 1247 (1995)). *clk-1* mutants also have an extended life-span. This pleiotropic alteration of developmental and behavioral timing defines the Clock (Clk) phenotype, also exhibited by worms carrying mutations in any one of the genes *clk-2*, *clk-3* and *gro-1* (A. Wong et al., *Genetics* **139**, 1247 (1995); S. Hekimi et al., *Genetics* **141**, 1351 (1995)). Mutations in these four genes interact genetically to affect developmental rate and longevity (B. Lakowski et al., *Science* **272**, 1010 (1996)).

Many of the phenotypes of *clk-1* mutant worms result from changes in processes believed to be controlled by discrete biological clocks (A. Wong et al., *Genetics* **139**, 1247 (1995)). These procedures have widely different periods, from the sub-second time scale to hours, and were not previously thought to be connected in any mechanistic way (A. Wong et al., *Genetics* **139**, 1247 (1995)). We have speculated that *clk-1* mutations affect the function of a central bio-

logical clock that would ensure control, and temporal coordination, of these different processes, and govern the response of the organism to changes in temperature (A. Wong et al., *Genetics* **139**, 1247 (1995)). One key feature of the *clk-1* phenotype is that *clk-1* mutations exhibit a maternal effect: homozygous mutant (*clk-1/clk-1*) progeny from a heterozygous hermaphrodite (*clk-1/+*) are phenotypically wild type; only homozygous mutants from a homozygous mother exhibit a Clk phenotype. The maternal rescue not only influences early events, such as embryonic development, but extends to adult phenotypes, such as defecation and longevity (A. Wong et al., *Genetics* **139**, 1247 (1995)). This, and other evidence (A. Wong et al., *Genetics* **139**, 1247 (1995); S. M. Jazwinski, *Science* **273**, 54 (1996)) suggests the existence of a pervasive timing mechanism, a "clock", whose intrinsic rate is determined, or "set", early in development and that subsequently influences diverse timed processes throughout the worm's life.

It would be highly desirable to be provided with *clk-1* and to show that *clk-1* gene complements the *clk-1* phenotype and restores normal longevity.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide *clk-1* and to show that the *clk-1* gene complements the *clk-1* phenotype and restores normal longevity.

In accordance with the present invention there is provided the *clk-1* gene which complements the *clk-1* phenotype and restores normal longevity.

The cloned *clk-1* gene should allow us to better understand its biological function and its potential experimental or pharmaceutical uses. It should also allow us to identify other genes related to *clk-1*. If human *clk-1* gene is altered in transformed cells or

cell lines, it would indicate that it is involved in cancer and could be used for cancer diagnosis.

In accordance with the present invention there is provided a *clk-1* gene which has a function at the level of cellular physiology involved in developmental rate and longevity, wherein *clk-1* mutants have a longer life and a altered cellular metabolism relative to the wild-type.

In accordance with the present invention there is provided a method for the diagnosis and/or prognosis of cancer in a patient, which comprises the steps of:

a) obtaining a tissue sample from said patient;

b) analyzing DNA of the obtained tissue sample of step a) to determine if the human *clk-1* gene is altered, wherein alteration of the human *clk-1* gene is indicative of cancer.

In accordance with the present invention there is provided a mouse model of longevity, which comprises a gene knock-out of murine *clk-1* gene.

In accordance with the present invention there is provided a method to increase life span of an animal or a patient, which comprises the steps of down-regulating the expression of the *clk-1* gene and/or homologues thereof.

In accordance with the present invention there is provided a method of treatment of pathological conditions causing slow down of physiological rate of tissue and/or organ in a patient, which comprises administering an agent to said patient to promote tissue and/or organ specific overexpression of *clk-1* gene to increase the physiological rate.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the cloning of *clk-1*;

Fig. 2 illustrates the extension of life-span exhibited by *clk-1(e2519)* is rescued by the presence of

extrachromosomal arrays containing the wild-type *clk-1* gene;

Fig. 3A illustrates the alignment of the nematode CLK-1 (SEQ ID NO:13), rat COQ7 (SEQ ID NO:14) and yeast Cat5p/Coq7p sequences (SEQ ID NO:17), together with the partial sequence of murine (SEQ ID NO:15) and human CLK-1 (SEQ ID NO:16) homologues;

Fig. 3B illustrates the duplication within the CLK-1 sequence (SEQ ID NO:13) and its homologues;

Fig. 4 illustrates that the nematode gene *clk-1* restores the ability of a yeast Δ*cat5/coq7* null mutant to utilize glycerol as a carbon source;

Fig. 5 illustrates the comparison of the TOC-1 *C. elegans* sequence (SEQ ID NO:18) with homologous sequences in humans (SEQ ID NO:19) and mice (SEQ ID NO:20);

Fig. 6 illustrates the schematic of the expression construct pPD9577.2op;

Fig. 7 illustrates the defecation cycle lengths of individual animals of three genotypes; and

Fig. 8 illustrates the survival over time of the wild type (N2) and animals expressing CLK-1::GFP and carrying two wild type chromosomal copies of clk-1.

DETAILED DESCRIPTION OF THE INVENTION

Mutations in the *Caenorhabditis elegans* gene *clk-1* affect biological timing and extend longevity. In accordance with the present invention, we report the identification of *clk-1* and show that the cloned gene complements the *clk-1* phenotype and restores normal longevity. CLK-1 is conserved among eukaryotes, including humans, and is structurally similar to the yeast metabolic regulator Cat5p/Coq7p. These proteins contain a tandem duplication of a core 82-residue (TRC) domain. *clk-1* complements the Δ*cat5/coq7* mutant pheno-

type, demonstrating that *clk-1* and *CAT5/COQ7* share biochemical function and that *clk-1* acts at the level of cellular physiology. Furthermore, this supports the idea that animal aging has a basis in cellular metabolism.

clk-1 lies on linkage group III, between the genes *dpy-17* and *lon-1* (A. Wong et al., *Genetics* **139**, 1247 (1995); S. Hekimi et al., *Genetics* **141**, 1351 (1995); Fig. 1). Top line shows the genetic map in the *clk-1* region. *clk-1* had been previously mapped to this region (A. Wong et al., *Genetics* **139**, 1247 (1995); S. Hekimi et al., *Genetics* **141**, 1351 (1995)) and we refined its location to give the map position shown in Fig. 1.

Some cosmids tested for rescuing activity are shown here (C. Mello and A. Fire, *Meth. Cell Biol.* **48**, 451 (1995)). Stable rescue that persisted for many generations was obtained only with ZC400. + indicates rescue of all phenotypes; ± indicates rescue of just the defecation phenotype; - indicates no rescue. pRA41 is a derivative of ZC400 with an internal *Sac* I deletion. The insert in pRA41 contains three predicted genes, ZC395.10, ZC395.3 and ZC395.2 (ACeDB; F. H. Eeckman et al., *Meth. Cell Biol.* **48**, 583 (1995)). A deletion in ZC395.2 eliminates *clk-1* rescuing activity, while pRA40 rescues *clk-1*, indicating that ZC395.2 is the *clk-1* gene. S = *Sac* I X = *Xba* I, E = *Eco* RI. The nematode EST CEESX93F (ACeDB; F. H. Eeckman et al., *Meth. Cell Biol.* **48**, 583 (1995)) matches the 3' end of *toc-1*.

We have first described that the *clk*-1 gene is part of an operon of two genes, *toc*-1 and *clk*-1. However, we had few details about the protein (TOC-1) encoded by *toc*-1, except that it is somewhat similar to members of a family of divalent cation transporting

transmembrane transporters, the CDF family (Paulsen I.T. and Saier M.H., J. Membr. Biol., **156**:99-103 (1997)). We have now identified by database searches a true human homologue of toc-1 which we call htoc-1. We have used several database entries (Genbank accessions: AA214573, AA134438, AA134439, AA213498, AA505430) to obtain a consensus sequence which predicts a protein with high homology to TOC-1 (Fig. 5). Identical residues are shown in bold and are indicated by a dot above the residue. For humans the middle part of the sequence is not yet available and for mice only the C-terminal part of the sequence is available at this time.

A homologous mouse sequence (mtoc-1) was also established from database entries (Genbank accessions: AA03268A, AA033329, AA097624) and is also provided in Fig. 5.

We resequenced *clk-1* from *e2519*, *qm30* and *qm51*. *e2519*, a G→A transition introduces a new *Hin*d III site, and causes an E→K missense alteration in an absolutely conserved residue (see Fig. 3). *qm30* results in a 590-bp deletion, starting 12 nucleotides 3' of the lesion in *e2519*, and encompassing the entire last exon. *qm51* alters the absolutely conserved terminal G in the intron 2 splice acceptor. Sequencing of allele *qm11*, which has a phenotype essentially identical to *e2519*, revealed an identical lesion. The low probability of independently obtaining the same mutation twice suggests that the original allele was lost.

Overlapping cosmids from the candidate region (Fig. 1) were assayed for their ability to rescue the mutant phenotype on micro-injection into *clk-1* mutants. The cosmid ZC400 was found to rescue the mutant phenotype of both strong (*qm30*) and weaker (*e2519*) *clk-1* alleles, and fully recapitulated the maternal effect.

Over a number of generations (10-20), ZC400-containing extrachromosomal arrays lost their ability to rescue fully the slow growth phenotype of *clk-1* mutants, but the rescue of slow defecation persisted. This loss of rescue of the developmental phenotype probably reflects a ubiquitous phenomenon in *C. elegans*, where transgenic arrays undergo transcriptional silencing of arrays in the germline and in early embryos, possibly because of their complex repeated structure (C. Mello and A. Fire, *Meth. Cell Biol.* **48**, 451 (1995)). If so, it implies that later zygotic expression of *clk-1*(+) is sufficient to rescue adult behavioral defects, but maternal or early zygotic expression is needed to rescue slow development.

Injection of ZC400 subclones localized *clk-1* to a 1.9 kb *Eco* RI fragment. Extrachromosomal arrays containing this fragment restored developmental and behavioral rates to wild-type speed for at least one generation. Stably transformed lines, however, showed no amelioration of the slow-growth phenotype, but we observed that as adults these worms defecated at a wild-type rate. This fragment is predicted to contain a single gene, ZC395.2, which is altered in three *clk-1* alleles, thereby confirming the identity of this gene as *clk-1* (Fig. 1). The weaker *clk-1* allele is a missense mutation; the stronger ones involve the disruption of entire exons.

We previously demonstrated that all phenotypes of *clk-1* mutants can be fully maternally rescued, that all alleles exhibit the same pattern of phenotypes and that all alleles fail to complement each other for all phenotypes. Given this, the molecular evidence presented here unequivocally established that mutations in the *clk-1* gene are responsible for all of the phenotypes seen in *clk-1* mutant worms.

The *clk-1* gene lies just downstream of the predicted gene, ZC395.3 (Fig. 1). Using reverse transcription PCR, we established the 5' and 3' ends of both genes and their splicing patterns. We found that *clk-1* is exclusively *trans*-spliced (D.A. Zorio et al., *Nature* **372**, 270 (1994); J. Spieth et al., *Cell* **73**, 521 (1993)) to the splice leader SL2 at its 5' end, while the upstream gene is *trans*-spliced to SL1, in both cases immediately upstream of the initiator AUG codon. The 3' end of the upstream genes lies 105 bp from the 5' end of *clk-1*. The pattern of *trans*-splicing and the intergenic distance are typical of genes organized into operons in *C. elegans* (T. Blumenthal, *Trends Genet.* **11**, 132 (1995)), suggesting that the two genes share a promoter 5' of the upstream gene. The introns in *clk-1* were correctly predicted, but the real product of ZC395.3 (Fig. 1C) lacks the first predicted exon. This gene potentially encodes a protein that has similarity to a family of divalent metal ion transporters, so we have named it *toc-1*, for transporter-like protein in an operon with *clk-1*. Although there are cases of functionally related genes occurring together in operons in *C. elegans* and being coordinately expressed (T. Blumenthal, *Trends Genet.* **11**, 132 (1995)), there is no obvious functional relationship between *clk-1* (see below) and *toc-1*.

One phenotype of *clk-1* mutants is their extended life-span. To demonstrate that the lesion in *clk-1(e2519)* was responsible for the mutant worms' extended longevity, rather than it resulting from a difference in genetic background, we assayed the longevity of fully-rescued *e2519* mutant worms carrying the ZC400-containing extrachromosomal array *qmEx109*, as well as those carrying *qmEx96* extrachromosomal array containing the *clk-1* 1.9 kb *Eco* RI fragment.

As adults, *e2519; qmEx96* worms show full rescue of their defecation cycle even though their development is slow. We found that the presence of either *qmEx96* or *qmEx109* restores a wild-type life-span to *e2519* mutant worms. The life-span of *e2519; qmEx109* worms was indistinguishable from that of N2 worms (Fig. 2). The extension of life-span exhibited by *clk-1(e2519)* is rescued by the presence of an extrachromosomal array containing the wild-type *clk-1* gene. Graphs show the percentage of worms alive on a given day after being laid as eggs during a 2.5 hour period on day 0; N2 (□), *clk-1(e2519)* (●), *e2519; qmEx109* (Δ) (Fig. 2). For *e2519; qmEx96* (Δ) a 6-hour limited hatching was used. The mean life-spans, with standard errors, are 20.4±0.8, 28.1±1.4, 20.2±0.9 and 20.4±0.7 days, respectively. The worms were maintained at 18°C throughout and their longevity scored as previously described (B. Lakowski et al., *Science* **272**, 1010 (1996)). Sample size is 50 worms of each genotype, except for *e2519; qmEx109* which is 48.

The *clk-1* gene is predicted to encode a 187-residue protein, CLK-1, that is similar to the product of the *Saccharomyces cerevisiae* gene *CAT5/COQ7* (Cat5p/Coq7p; M. Proft et al., *EMBO J.* **14**, 6116 (1995); B. N. Marbois et al., *J. Biol. Chem.* **271**, 2995 (1996)). A rat homolog of Cat5p/Coq7p has also been described (T. Jonassen et al., *Arch. Biochem. Biophys.* **330**, 285 (1996)). The three proteins are 33% identical over 177 residues, although their N-termini show no similarity, either in terms of length or composition (Fig. 3A). CLK-1 is highly conserved between nematodes, yeast and rodents. Over the length of the rat protein, the identity between CLK-1 and its yeast and rat homologues is 42% and 53%, respectively. Introduced gaps are marked by dashes. Reduction-of-function alleles of

both *clk-1* and *CAT5/COQ7* are known, and both occur in absolutely conserved residues (indicated by arrows here, boxed in B, below); G→D in *coq7-1* (B. N. Marbois et al., *J. Biol. Chem.* **271**, 2995 (1996)) and E→K in *e2519*. The rat sequence (GenBank accession no. U46149) appears to contain sequencing errors in the vicinity of residues 82-84 and 151-154 (marked by dots). Using the sequence of the rat gene, we were able to identify and partially sequence murine and human homologues of *clk-1*.

Over the available predicted sequences of 43 and 126 amino acids, the human and mouse proteins are 93% and 97% identical to the rat protein, respectively (Fig. 3). These five proteins are unrelated to any other known sequence, and there are few indications as to their biochemical function.

The protein sequences can each be split and aligned to reveal the presence of an 82-residue tandemly-repeated core domain, which we call the TRC domain, for tandemly-repeated in CLK-1 (or Cat5p/Coq7p or rat COQ7; Fig. 3B). Each of the sequences shown in (Fig. 3A) can be split and aligned to reveal the presence of a tandemly repeated TRC domain. There is a single site of insertions for both N- and C-terminal domains; these have been removed for this alignment, as marked by the small black dots. Those residues identical in four or more of the six domains are shown in black lettering, those that are similar in four or more of the domains are shown in dark gray. Positions where there is absolute conservation of a hydrophobic residue are marked by a ϕ.

Overall, for all repeats, residues are absolutely conserved at 8 positions, and at an additional 12 positions all residues are similar. For each protein, its two TRC domains are juxtaposed without any

linking sequence. For each domain, there appears to be only a single point at which insertions are tolerated, flanked by regions predicted to be helical (Fig. 3B). Within these helical regions, (residues 34-56 and 116-144 for CLK-1) the spacing of conserved hydrophobic residues is suggestive of an interface for protein-protein interaction, such as a surface for dimerization (Fig. 3B). The two-domain structure seen in the proteins' primary structure is expected to be reflected by two equivalent domains at the level of the proteins' tertiary structure; it is likely that these proteins evolved from a precursor that contained just one domain, but that was able to homodimerize.

The two strongest *clk-1* alleles (A. Wong et al., *Genetics* **139**, 1247 (1995)) are *qm30*, a 590 bp deletion that eliminates 35 codons, including the entire last exon, together with all of the 3' untranslated region of the gene, and *qm51*, a point mutation that eliminates the splice acceptor site of the second intron (Fig. 1). The less severe allele *e2519* is a point mutation that results in a glutamic acid to lysine change (E148K; Figs. 1 & 3). As the phenotypes measured in *e2519* worms are quantitatively intermediate between wild type and *qm30*, there would appear to be a correlation between the severity of the Clk phenotype and the amount of residual *clk-1* gene activity, relative to that in wild-type worms.

What is the function of the yeast homolog *CAT5/COQ7* ? Wild type yeast grow most rapidly with glucose as a carbon source. When glucose is present, the expression of many genes, including those involved in gluconeogenesis, is strongly repressed. When yeast are transferred to a non-fermentable carbon source, such as glycerol or ethanol, the derepression of *PCK1*, which encodes the gluconeogenic enzyme phospho-enolpyruvate

carboxykinase, requires the activity of *CAT5/COQ7* (M. Proft et al., *EMBO J.* **14**, 6116 (1995)). This derepression of *PCK1* is mediated by a carbon source-responsive element (CSRE) in its promoter (M. Proft et al., *EMBO J.* **14**, 6116 (1995)). As well as being necessary for the formation of a specific CSRE transcriptional activation complex, *CAT5/COQ7* appears to be involved in the control of expression of other enzymes of gluconeogenesis, and those of respiration and the glyoxylate cycle (M. Proft et al., *EMBO J.* **14**, 6116 (1995)). But its role in all these processes appears to be indirect and likely part of a complex regulatory mechanism. For example, *CAT5/COQ7* is subject to partial glucose repression, and its expression under derepressing conditions requires the activities of *CAT1/SNF1* and *CAT8* as well as *CAT5/COQ7* itself (M. Proft et al., *EMBO J.* **14**, 6116 (1995)).

CAT5/COQ7 has also been characterized as being involved in ubiquinone (coenzyme Q) biosynthesis (B. N. Marbois et al., *J. Biol. Chem.* **271**, 2995 (1996)). *cat5/coq7* mutants do not synthesize this lipid-soluble two-electron carrier, which is essential for non-fermentative growth. Chemical characterization of the ubiquinone biosynthetic intermediates in a yeast strain with a point mutation in *CAT5/COQ7* (B. N. Marbois et al., *J. Biol. Chem.* **271**, 2995 (1996); Fig. 3) revealed the accumulation of 5-demethoxyubiquinone, a late intermediate in the biosynthetic pathway. Strains in which *CAT5/COQ7* was deleted, however, are reported to accumulate 3-hexaprenyl-4-hydroxybenzoic acid, an earlier intermediate (B. N. Marbois et al., *J. Biol. Chem.* **271**, 2995 (1996)). Thus *CAT5/COQ7* appears to control ubiquinone synthesis at two or more steps, although its mode of action is obscure. The pleiotropic effects of mutation of *CAT5/COQ7* have led to the proposal that

there is a co-regulation of respiratory chain components, the biogenesis of mitochondria, and gluconeogenesis, with *CAT5/COQ7* being a likely link connecting glucose derepression with respiration (M. Proft et al., *EMBO J.* **14**, 6116 (1995)). Thus *CAT5/COQ7* appears to be important in the regulation of multiple parallel processes of metabolism. This is consistent with our view of *clk-1* regulating many disparate physiological and metabolic processes in *C. elegans*.

To test whether the structural similarity extended to functional equivalence, we constructed a Δ *cat5/coq7* yeast strain, which as expected (M. Proft et al., *EMBO J.* **14**, 6116 (1995); B. N. Marbois et al., *J. Biol. Chem.* **271**, 2995 (1996)) failed to grow on glycerol.

All yeast manipulations were in the SEY6210 background (*MATa, leu2-3, ura3-52, his3-Δ200, lys2-801, trp1-Δ901, suc2-Δ9*). Yeast cells were grown under standard conditions (YNB, YEPD and YEPG). Strains were transformed using the lithium acetate procedure with sheared, denatured carrier DNA. The *CAT5/COQ7* locus was disrupted using a PCR-mediated approach (the primers used were ML134 and ML135). The *CAT5/COQ7* gene was entirely replaced with a DNA fragment containing a disruption module encoding the Green Fluorescent Protein and the *HIS3* gene (R. K. Niedenthal et al., *Yeast* **12**, 773 (1996)). Haploid cells were transformed with the PCR product and *HIS3* integrants were selected on minimal medium lacking histidine. Gene disruptions were confirmed by PCR analysis using primers ML136, ML137 and ML138. The Δ*cat5/coq7* strain failed to grow on YEPG, or $YEPE_3$ which contains ethanol (M. Proft et al., *EMBO J.* **14**, 6116 (1995)). The primer used were ML134, TTTTCATATACGGGATTTTCAGGAAAAAAAACAATAGAAATCTATAAAACATGAGTAAAGGAGAAGAAC (SEQ ID NO:1); ML135, CCGTT-

TTCCTTTCAATTCTCCTTTTCTGGCATAACGCGACTGATGTATGCCACGCGCGCC TCGTTCAGAATG (SEQ ID NO:2); ML136, CGTACTCTGTCTATAT-TTCCC (SEQ ID NO:3); ML137, GCGTTAAAATGCGTAAGGATG (SEQ ID NO:4); ML138, CCACTTGCCACCTATCACC (SEQ ID NO:5).

Introduction of a multicopy plasmid containing the *C. elegans clk-1* coding sequence, within an expression cassette which includes the constitutive promoter and 3' sequence of the *ADH1* gene, conferred the ability to grow on glycerol to the Δ*cat5/coq7* strain (Fig. 4). A wild-type strain (A. Wong et al., *Genetics* **139**, 1247 (1995)) or yeast cells harboring a deletion of the *CAT5/COQ7* gene and containing plasmid pVT102-U (multicopy and constitutive *ADH1* promoter) with a *CAT5/COQ7* insert (S. Hekimi et al., *Genetics* **141**, 1351 (1995)), *clk-1* insert (B. Lakowski et al., *Science* **272**, 1010 (1996)) or alone (S. M. Jazwinski, *Science* **273**, 54 (1996)) were tested for growth on YEP plates containing 3% glycerol, and incubated at 30°C for seven days. Transformants were grown on selective medium containing 2% glucose prior to streaking.

The *CAT5/COQ7* locus was directly amplified from yeast genomic DNA by PCR using *Pfu* polymerase (Stratagene) and primers SHP69 and SHP70. A cDNA corresponding to the entire *clk-1* coding sequence was obtained by PCR amplification, also with *Pfu* polymerase, and nested primer pairs SHP57/SHP59 and SHP57/SHP58 on single-stranded cDNA that had been synthesized by priming with SHP59. The respective yeast and nematode PCR products were digested with *Hin*d III and ligated to *Hin*d III-cut and dephosphorylated pVT102-U (T. Vernet et al., *Gene* **52**, 225 (1987)). They were separately transformed into competent TG1 cells and the desired recombinant plasmids recovered and each re-transformed into the Δ*cat5/coq7* strain. As well as restoring growth on glycerol (Fig. 4), both the *CAT5/COQ7* and

clk-1 containing plasmids restored the ability of the Δ *cat5/coq7* strain to grow on ethanol ($YEPE_3$ medium). The primers used were SHP57, CAGAAGCTTCCCAGTTACTCAA-GATGTTCCG (SEQ ID NO:6); SHP58, CAGAAGCTTTGTTCAAAT-TTTCTCAGC (SEQ ID NO:7); SHP59, ATGGAAGAAAAGGGACAC (SEQ ID NO:8); SHP69, CAGAAGCTTCTATAAAACATGTTTCC (SEQ ID NO:9); SHP70, CAGAAGCTTAAATTCTTTCGGCACTCC (SEQ ID NO:10).

This functional complementation of the Δ*cat5/coq7* null by *clk-1* is consistent with the reported functional complementation of the yeast mutant by the rat homolog (T. Jonassen et al., *Arch. Biochem. Biophys.* **330**, 285 (1996)) and is indicative of a common biochemical function for these three genes. In spite of their common biochemical function and roles in regulatory mechanisms, one must be cautious in attempting to understand the physiological defects seen in *clk-1* worms in terms of the phenotypic defects of *cat5/coq7* mutant strains since yeast have some highly specialized systems of metabolic regulation not seen in most other eukaryotes. We are currently investigating, however, whether glucose metabolism and ubiquinone biosynthesis are affected in *clk-1* mutants. Nevertheless, the interspecific functional complementation raises the possibility that a central mechanism of metabolic coordination, which regulates distinct downstream regulators, is conserved in all eukaryotes, including humans.

In accordance with the present invention, we have shown that *clk-1* affects the longevity of *C. elegans*. The functional complementation of Δ *cat5/coq7* by *clk-1* in yeast cells, however, highlights one other aspect of the character of this gene: it acts at the level of a single cell (a phenotype previously noted in single-celled *C. elegans* eggs; A. Wong et al.,

Genetics **139**, 1247 (1995)). A number of observations suggest that the cellular equivalent of organismal aging is a limit to proliferative capacity (known as cellular senescence; reviewed in J. Campisi, *Cell* **84**, 497 (1996); L. Guarente, *Cell* **86**, 9 (1996)). Consequently it will be of interest to determine whether Δ *cat5/coq7* mutants live longer than wild-type yeast cells.

In conclusion, we have identified the *C. elegans* gene *clk-1* and shown that it is structurally and functionally homologous to a yeast central metabolic regulator. This supports our previous speculation that the long life of *clk-1* mutants might be a consequence of slower cellular metabolism, with an attendant reduction in the rate of production of detrimental by-products (B. Lakowski et al., *Science* **272**, 1010 (1996)). Our findings also lend support to the idea that multicellular organisms age because their cells age (J. Campisi, *Cell* **84**, 497 (1996); L. Guarente, *Cell* **86**, 9 (1996)).

The present invention will be more readily understood by referring to the following example which is given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Reporter gene expressing a CLK-1::GFP fusion protein

We have also constructed a reporter gene expressing a fusion protein containing the entire CLK-1 amino acid sequence fused at the C-terminal end to green fluorescent protein (GFP). The reporter gene expresses the protein in the context of the *clk*-1 operon (Fig. 6).

This construct utilized the vector pPD95.77 (gift from Dr. Andrew Fire) which was designed to allow a protein of interest to be transcriptionally fused to

Green Fluorescent Protein (GFP). In this case, the *clk*-1 operon (including a 5' upstream region presumably containing the full promoter as it extends to the next predicted gene, *toc*-1, *clk*-1 and the *toc*-1/*clk*-1 intergenic region) was amplified by the polymerase chain reaction (PCR) and cloned into pPD95.77. The primers used were SHP121 (AAAAGGGTCGACCGAAAAGAGAACTAGACGG (SEQ ID NO:11)) and SHP122 (AAGGGGATCCAATTTTCTCAGCAATCGC (SEQ ID NO:12)). These primers had restriction sites, SalI and BamHI respectively, built into their 5' ends to allow the directed cloning of the amplified product. In the figure, exonic DNA is represented by boxes; shading indicates coding sequence. The *clk*-1 gene is included in its entirety, with the exception of the termination codon which was excluded to allow translation through *gfp*. The hashed region in the figure between *clk*-1 and *gfp* is part of the vector's multiple cloning site (102 base pairs) and codes for 34 amino acids which provide a linker between CLK-1 and GFP.

The reporter construct fully rescues the phenotype of a *clk*-1(qm30) mutant upon injection and extrachromosomal array formation, indicating that the fusion to the GFP moiety does not significantly inhibit the function of CLK-1. We found that *clk*-1 is expressed in all cells at all stages. Furthermore, fluorescence microscopy showed that the CLK-1::GFP fusion protein is localized to mitochondria. The localization was confirmed by co-localization with a mitochondrion-specific vital dye, G6-rhodamine.

The reporter gene was also injected into wild type worms and individuals carrying extrachromosomal arrays were examined for phenotypic alterations. Presumably, the expression of CLK-1::GFP from the extrachromosomal array together with the expression of normal CLK-1 from the endogenous wild type gene corre-

sponds to an overexpression of *clk*-1 activity. As a measure of physiological rates, we examined the length of the defecation cycle in aging worms. The defecation cycle was examined at two times (day 1 and day 4 of adulthood) in three genotypes (wild type, *clk*-1(qm30) and animals expressing CLK-1::GFP in a wild type background (Fig. 7)).

Five inter-defecation periods were scored for each animal and the means calculated and plotted. Animals were maintained at 20°C throughout the experiment. The cycle lengths are plotted in 2 seconds intervals.

We found that, as seen previously, the rate of defecation of clk-1 mutants at day 1 is much slower on average than that of wild type animals. However, on day 4 there was no significant difference between these two genotypes (Fig. 7 top and middle panels). This suggests that the normal slowing down of defecation between day 1 and day 4 in the wild type is due to down-regulation of *clk*-1. Furthermore, we found that a significant proportion of the animals overexpressing the *clk*-1 activity do not have slow defecation on day 4 (Fig. 7 bottom panel). It should be remembered that the animals which carry a free extrachromosomal array are mosaic, which could explain the variability of the effect observed. Together these observations suggest that a downward regulation of the activity of *clk*-1 underlies a slowing down of physiological rates during aging which can be prevented by an artificial increase of *clk*-1 activity.

We have previously argued that *clk*-1 mutant animals live longer because their physiological rates are reduced. We have now observed that the defecation rate of animals overexpressing *clk*-1 appears to be increased over the wild type in aging animals (4 days of adulthood). The defecation represents only one measure of

the physiology of the worms but it is likely that the observed increase reflects a general increase in physiological rates. We have therefore examined the life span of animals overexpressing *clk*-1 (animals expressing CLK-1::GFP in a wild type background). We found that these animals indeed live a significantly shorter life than the wild type (Fig. 8).

Animals were maintained at 20°C throughout the experiment. The sample size was 100.

These new results suggest that the level of *clk*-1 activity controls physiological rates as well as life span. A reduced level of *clk*-1 activity as found in mutants leads to slower physiological rates and an increased life span, while an increased activity leads to faster physiological rates (as seen on day 4 of adulthood) and a shortened life span. It suggests that artificially down-regulating the expression of the *clk*-1 gene or its homologues in others organisms (for example by anti-sense therapy or pharmacological means) would lead to an increased life span. If artificial down-regulation could be targeted to a particular tissue or organ, it could lead to a specific physiological slowing-down of this tissue or organ and a concomitant slower rate of degradation by the aging process. Alternatively, the tissue- or organ-specific overexpression of *clk*-1 could allow one to increase the physiological rate of tissues or organs whose functional rates are slowed because of a pathological condition.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and

including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: McGILL UNIVERSITY et al.

(ii) TITLE OF THE INVENTION: STRUCTURAL AND FUNCTIONAL CONSERVATION OF THE C. ELEGANS CLOCK GENE clk-1

(iii) NUMBER OF SEQUENCES: 20

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/028,977
(B) FILING DATE: 21-OCT-1996

(A) APPLICATION NUMBER: 60/033,196
(B) FILING DATE: 18-DEC-1996

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTTTCATATA CGGGATTTTC AGGAAAAAAA ACAATAGAAA TCTATAAAAC ATGAGTAAAG 60
GAGAAGAAC 69

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGTTTTCCT TTCAATTCTC CTTTTCTGGC ATAACGCGAC TGATGTATGC CACGCGCGCC 60
TCGTTCAGAA TG 72

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGTACTCTGT CTATATTTCC C 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGTTAAAAT GCGTAAGGAT G 21

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCACTTGCCA CCTATCACC 19

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAGAAGCTTC CCAGTTACTC AAGATGTTCC G 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGAAGCTTT GTTCAAATTT TCTCAGC 27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGGAAGAAA AGGGACAC 18

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAGAAGCTTC TATAAAACAT GTTTCC 26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAGAAGCTTA AATTCTTTCG GCACTCC 27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAAAGGGTCG ACCGAAAAGA GAACTAGACG G 31

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AAGGGGATCC AATTTTCTCA GCAATCGC 28

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 187 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
Met Phe Arg Val Ile Thr Arg Gly Ala His Thr Ala Ala Ser Arg Gln
 1               5                  10                  15
Ala Leu Ile Glu Lys Ile Ile Arg Val Asp His Ala Gly Glu Leu Gly
            20                  25                  30
Ala Asp Arg Ile Tyr Ala Gly Gln Leu Ala Val Leu Gln Gly Ser Ser
        35                  40                  45
Val Gly Ser Val Ile Lys Lys Met Trp Asp Glu Glu Lys Glu His Leu
    50                  55                  60
Asp Thr Met Glu Arg Leu Ala Ala Lys His Asn Val Pro His Thr Val
65                  70                  75                  80
Phe Ser Pro Val Phe Ser Val Ala Ala Tyr Ala Leu Gly Val Gly Ser
                85                  90                  95
Ala Leu Leu Gly Lys Glu Gly Ala Met Ala Cys Thr Ile Ala Val Glu
            100                 105                 110
Glu Leu Ile Gly Gln His Tyr Asn Asp Gln Leu Lys Glu Leu Leu Ala
        115                 120                 125
Asp Asp Pro Glu Thr His Lys Glu Leu Leu Lys Ile Leu Thr Arg Leu
    130                 135                 140
Arg Asp Glu Glu Leu His His His Asp Thr Gly Val Glu His Asp Gly
145                 150                 155                 160
Met Lys Ala Pro Ala Tyr Ser Ala Leu Lys Trp Ile Ile Gln Thr Gly
                165                 170                 175
Cys Lys Gly Ala Ile Ala Ile Ala Glu Lys Ile
            180                 185
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 179 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Thr Leu Asp Asn Ile Asn Arg Ala Ala Val Asp Arg Ile Ile Arg
 1               5                  10                  15
Val Asp His Ala Gly Glu Tyr Gly Ala Asn Arg Ile Tyr Ala Gly Gln
            20                  25                  30
Met Ala Val Leu Gly Arg Thr Ser Val Gly Pro Val Ile Gln Lys Met
        35                  40                  45
Trp Asp Gln Glu Lys Asn His Leu Lys Lys Phe Asn Glu Leu Met Val
    50                  55                  60
Ala Phe Arg Val Arg Pro Thr Val Leu Met Pro Leu Trp Asn Val Ala
65                  70                  75                  80
Gly Phe Ala Leu Gly Ala Gly Thr Ala Leu Leu Gly Lys Glu Gly Gly
                85                  90                  95
Met Ala Cys Thr Val Ala Val Glu Glu Ser Ile Ala His His Tyr Asn
            100                 105                 110
```

```
Asn Gln Ile Arg Met Leu Met Glu Glu Asp Ala Glu Lys Tyr Glu Glu
          115                 120                 125
Leu Leu Gln Val Ile Lys Gln Phe Arg Asp Glu Glu Leu Glu His His
    130                 135                 140
Asp Thr Gly Leu Glu His Asp Ala Glu Leu Ala Pro Ala Tyr Thr Leu
145                 150                 155                 160
Leu Lys Arg Leu Ile Gln Ala Gly Cys Ser Ala Ala Ile Tyr Leu Ser
                165                 170                 175
Glu Arg Phe
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Lys Met Trp Asp Gln Glu Lys Asn His Leu Lys Lys Phe Asn Glu Leu
 1               5                  10                  15
Met Ile Ala Phe Arg Val Arg Pro Thr Val Leu Met Pro Leu Trp Asn
            20                  25                  30
Val Ala Gly Phe Ala Leu Gly Ala Gly Thr Ala Leu Leu Gly Lys Glu
        35                  40                  45
Gly Ala Met Ala Cys Thr Val Ala Val Glu Glu Ser Ile Ala Asn His
    50                  55                  60
Tyr Asn Asn Gln Ile Arg Met Leu Met Glu Glu Asp Pro Glu Lys Tyr
65                  70                  75                  80
Glu Glu Leu Leu Gln Val Ile Lys Gln Phe Arg Asp Glu Glu Leu Glu
                85                  90                  95
His His Asp Thr Gly Leu Asp His Asp Ala Glu Leu Ala Pro Ala Tyr
            100                 105                 110
Ala Leu Leu Lys Arg Ile Ile Gln Ala Gly Cys Ser Ala Ala Ile Tyr
        115                 120                 125
Leu Ser Glu Arg Phe
    130
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Lys Met Trp Asp Gln Glu Lys Asp His Leu Lys Lys Phe Asn Glu Leu
 1               5                  10                  15
Met Val Met Phe Arg Val Arg Pro Thr Val Leu Met Pro Leu Trp Asn
            20                  25                  30
```

```
Val Leu Gly Phe Ala Leu Gly Ala Gly Thr Ala Leu Leu Gly
        35                  40                  45
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 272 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Met Phe Pro Tyr Phe Tyr Arg Arg Glu Phe Tyr Ser Cys Glu Asn Val
 1               5                  10                  15
Val Ile Phe Ser Ser Lys Pro Ile Gln Gly Ile Lys Ile Ser Arg Ile
            20                  25                  30
Arg Glu Arg Tyr Ile Glu Ile Met Leu Ser Arg Val Ser Val Phe Lys
        35                  40                  45
Pro Ala Ser Arg Gly Phe Ser Val Leu Ser Ser Leu Lys Ile Thr Glu
    50                  55                  60
His Thr Ser Ala Lys His Thr Glu Lys Pro Glu His Ala Pro Lys Cys
65                  70                  75                  80
Gln Asn Leu Ser Asp Ala Gln Ala Ala Phe Leu Asp Arg Val Ile Arg
                85                  90                  95
Val Asp Gln Ala Gly Glu Leu Gly Ala Asp Tyr Ile Tyr Ala Gly Gln
            100                 105                 110
Tyr Phe Val Leu Ala His Arg Tyr Pro His Leu Lys Pro Val Leu Lys
        115                 120                 125
His Ile Trp Asp Gln Glu Ile His His His Asn Thr Phe Asn Asn Leu
    130                 135                 140
Gln Leu Lys Arg Arg Val Arg Pro Ser Leu Leu Thr Pro Leu Trp Lys
145                 150                 155                 160
Ala Gly Ala Phe Ala Met Gly Ala Gly Thr Ala Leu Ile Ser Pro Glu
                165                 170                 175
Ala Ala Met Ala Cys Thr Glu Ala Val Glu Thr Val Ile Gly Gly His
            180                 185                 190
Tyr Asn Gly Gln Leu Arg Asn Leu Ala Asn Gln Phe Asn Leu Glu Arg
        195                 200                 205
Thr Asp Gly Thr Lys Gly Pro Ser Glu Glu Ile Lys Ser Leu Thr Ser
    210                 215                 220
Thr Ile Gln Gln Phe Arg Asp Asp Glu Leu Glu His Leu Asp Thr Ala
225                 230                 235                 240
Ile Lys His Asp Ser Tyr Met Ala Val Pro Tyr Thr Val Ile Thr Glu
                245                 250                 255
Gly Ile Lys Thr Ile Cys Arg Val Ala Ile Trp Ser Ala Glu Arg Ile
            260                 265                 270
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 428 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Val Ser Phe Ser Trp Val Ser Arg Ser Leu His His Pro Gln Gly
 1               5                  10                  15
Lys Arg Gly Leu Ile Ser Thr Leu Ile Cys Leu Ala Cys Val Gly Ile
            20                  25                  30
Leu Ala Tyr Cys Val Ser Thr Ser His Ser Ile Val Leu Met Ser Thr
        35                  40                  45
Leu Trp Ile Thr Ile Phe Ser Phe Cys Ser Gln Phe Ala Ser Leu Tyr
    50                  55                  60
Ser Met Ser Ile Thr Glu Lys Pro Thr His Lys Phe Ser Tyr Gly Leu
65                  70                  75                  80
Ala Arg Val Pro Val Leu Ala Val Phe Ser Thr Thr Val Leu Ala Gln
                85                  90                  95
Leu Phe Ser Ile Phe Leu Ser Lys Glu Ser Phe Glu His Leu Leu Ser
            100                 105                 110
Pro Asp His His Gly Ser His Asp Ala Ser Ala Ala His Glu His Glu
        115                 120                 125
Val Glu Glu Ile Gly Gly Trp Pro Tyr Phe Val Gly Ser Ala Ala Ser
    130                 135                 140
Ser Val Ala Leu Leu Leu Ser Ala Tyr Ala Leu Lys Asn Gln Pro Phe
145                 150                 155                 160
Gln His Val Leu Gln Ser Ala Thr Ala Ser Ser Leu Gln Glu His Ala
                165                 170                 175
Ala Asp Leu Ser His Ala Val Cys Trp Val Ile Pro Gly Leu Ser Arg
            180                 185                 190
Leu Leu Leu Pro Arg Ile Asn Ser Met Val Leu Leu Ala Leu Thr Thr
        195                 200                 205
Thr Gly Leu Asn Leu Leu Cys Glu His Phe Lys His Asp Phe Ala Trp
    210                 215                 220
Ala Asp Pro Val Cys Cys Leu Leu Leu Ser Val Ala Val Phe Ser Thr
225                 230                 235                 240
Met Tyr Pro Leu Ser Thr Tyr Thr Gly Met Ile Leu Leu Gln Thr Thr
                245                 250                 255
Pro Pro His Leu Ile Asn Gln Ile Asp Arg Cys Ile Ser Glu Ala Ser
            260                 265                 270
His Ile Asp Gly Val Leu Glu Leu Lys Ser Gly Arg Phe Trp Gln Leu
        275                 280                 285
Asp Phe Asn Ser Leu Val Gly Thr Val Asp Val Arg Val Arg Arg Asp
    290                 295                 300
Ala Asp Glu Gln Asn Val Leu Ala His Val Thr Glu Lys Phe Ser Ser
305                 310                 315                 320
Val Ile Thr Val Leu Thr Val Gln Val Val Lys Asp Ala Ala Trp Ser
                325                 330                 335
Ala Gly Glu Gln Val Pro Tyr Ser Asn Gly His Ile His Lys Ser Glu
            340                 345                 350
Gly Asn His Ser His Asp Asn Gly His Gly His Ser His Asp His Asn
        355                 360                 365
Asp His Gly His Ser His Gly His Asp Asp His Gly His Asp Ser His
    370                 375                 380
Gly His Ser His Asp His Asn Glu His Asp His Gly His Ser His Gly
385                 390                 395                 400
Gly Asn Asn Asp Asn His Gly His Ser His Ser Ala Gly Ser Asp Asn
                405                 410                 415
His His Gly His Ser His Asp Gly Val Phe Tyr His
            420                 425
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 234 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Met Gly Thr Ile His Leu Phe Arg Lys Pro Gln Arg Ser Phe Phe Gly
 1               5                  10                  15
Lys Leu Leu Arg Glu Phe Arg Leu Val Ala Ala Asp Arg Ser Met Gly
            20                  25                  30
Arg Tyr Met Leu Phe Gly Val Ile Asn Leu Ile Cys Thr Gly Phe Leu
        35                  40                  45
Leu Met Trp Cys Ser Ser Thr Asn Ser Ile Ala Leu Xaa Ala Tyr Thr
    50                  55                  60
Tyr Leu Thr Ile Phe Asp Leu Phe Ser Leu Met Thr Cys Leu Ile Ser
65                  70                  75                  80
Tyr Trp Val Thr Leu Arg Lys Pro Ser Pro Val Tyr Ser Phe Gly Phe
                85                  90                  95
Glu Arg Leu Glu Val Leu Ala Val Phe Ala Ser Thr Val Leu Ala Gln
            100                 105                 110
Leu Gly Ala Leu Phe Ile Leu Lys Glu Ser Ala Glu Arg Xaa Leu Glu
        115                 120                 125
Gln Ser Xaa Leu Xaa Leu Cys Ile Pro Xaa Ser Val Tyr Ser Gly Lys
    130                 135                 140
Val Xaa Leu Gln Thr Thr Pro Pro His Val Ile Gly Gln Leu Asp Lys
145                 150                 155                 160
Leu Ile Arg Glu Val Ser Thr Leu Asp Gly Val Leu Glu Val Arg Asn
                165                 170                 175
Glu His Phe Trp Thr Leu Gly Phe Gly Ser Leu Ala Gly Ser Val His
            180                 185                 190
Val Arg Ile Arg Arg Asp Ala Asn Glu Gln Met Val Leu Ala His Val
        195                 200                 205
Thr Asn Arg Leu Tyr Thr Leu Val Ser Thr Leu Thr Val Gln Ile Phe
    210                 215                 220
Lys Asp Asp Trp Ile Arg Pro Gly Phe Thr
225                 230
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 183 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Tyr Pro Met Ser Val Tyr Ser Gly Lys Val Leu Leu Gln Thr Thr Pro
1 5 10 15
Pro His Val Ile Gly Gln Leu Asp Lys Leu Ile Arg Glu Val Ser Thr
20 25 30
Leu Asp Gly Val Leu Glu Val Arg Asn Glu His Phe Trp Thr Leu Gly
35 40 45
Phe Gly Ser Leu Ala Gly Ser Val His Val Arg Ile Arg Arg Asp Ala
50 55 60
Asn Glu Gln Met Val Leu Ala His Val Ser Asn Arg Leu Cys Thr Leu
65 70 75 80
Val Ser Thr Leu Thr Val Gln Ile Phe Lys Asp Asp Trp Ile Arg Pro
85 90 95
Ala Leu Ser Ser Gly Pro Val Ala Pro Asn Val Leu Asn Phe Ser Asp
100 105 110
His His Val Ile Pro Met Pro Leu Leu Lys Asn Val Asp Glu Arg Thr
115 120 125
Pro Val Thr Ser Thr Pro Ala Lys Pro Ser Ser Pro Ser Pro Glu Phe
130 135 140
Ser Phe Asn Thr Pro Gly Lys Asn Val Ser Pro Val Ile Leu Leu Asn
145 150 155 160
Thr Gln Thr Arg Pro Tyr Ser Leu Gly Leu Asn Arg Gly His Thr Pro
165 170 175
Tyr Ser Ser Val Phe Ser Gln
180

I CLAIM:

1. A *clk-1* gene which has a function at the level of cellular physiology involved in developmental rate and longevity, wherein *clk-1* mutants have a longer life and a altered cellular metabolism relative to the wild-type.

2. A method for the diagnosis and/or prognosis of cancer in a patient, which comprises the steps of:

a) obtaining a tissue sample from said patient;

b) analyzing DNA of the obtained tissue sample of step a) to determine if the human *clk-1* gene is altered, wherein alteration of the human *clk-1* gene is indicative of cancer.

3. A mouse model of longevity, which comprises a gene knock-out of murine *clk-1* according to claim 1.

4. A method to increase life span of an animal or a patient, which comprises the steps of downregulating the expression of the *clk-1* gene of claim 1 and/or homologues thereof.

5. A method of treatment of pathological conditions causing slow down of physiological rate of tissue and/or organ in a patient, which comprises administering an agent to said patient to promote tissue and/or organ specific overexpression of *clk-1* in to increase the physiological rate.

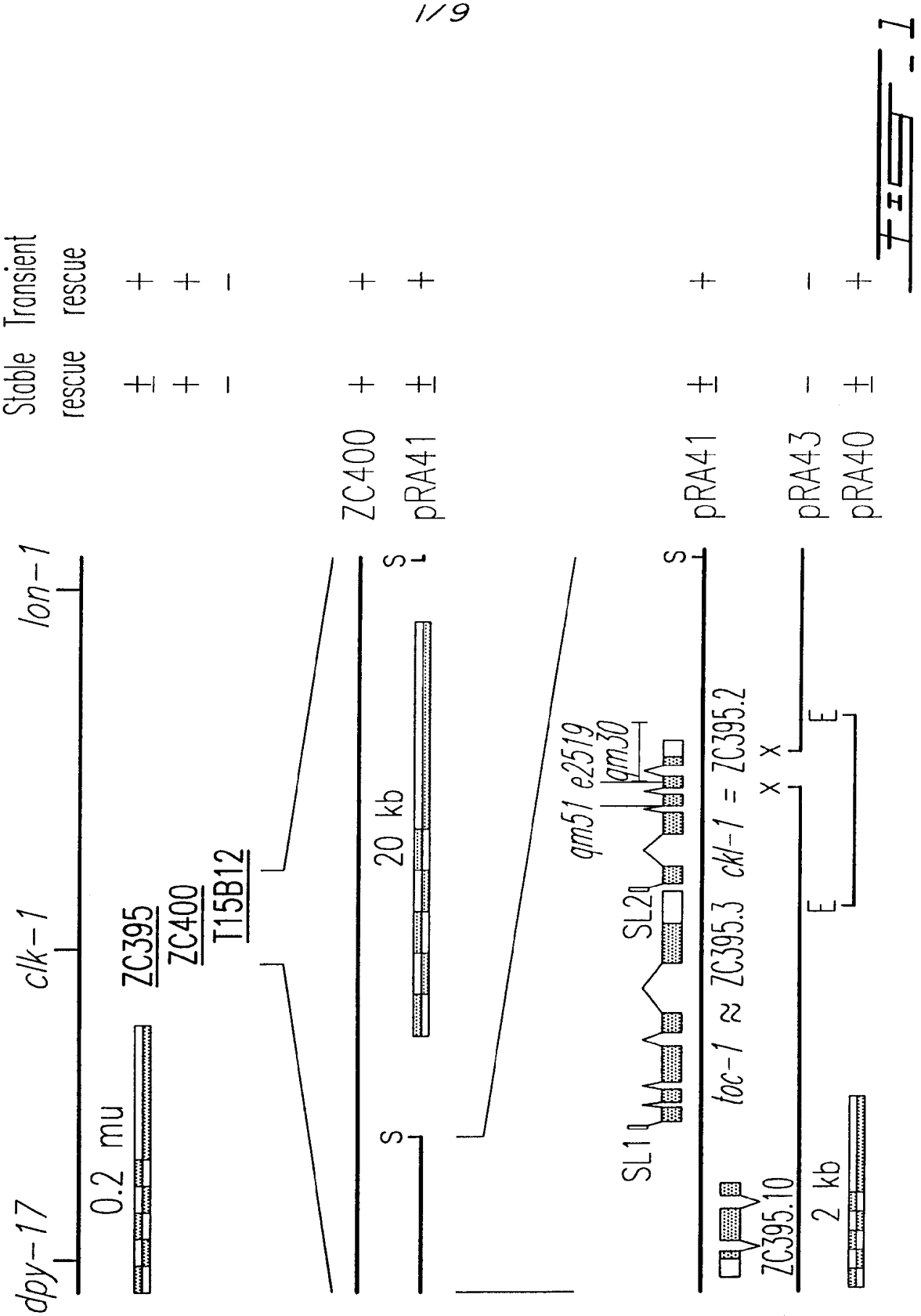
Stable rescue
Transient rescue
dpy-17
clk-1
lon-1
0.2 mu
ZC395
ZC400
T15B12
20 kb
S
S
ZC400
pRA41
qm51 e2519
qm30
SL2
SL1
toc-1 ≈ ZC395.3 ckl-1 = ZC395.2
X X
E
E
S
pRA41
pRA43
pRA40
ZC395.10
2 kb

FIG - 1

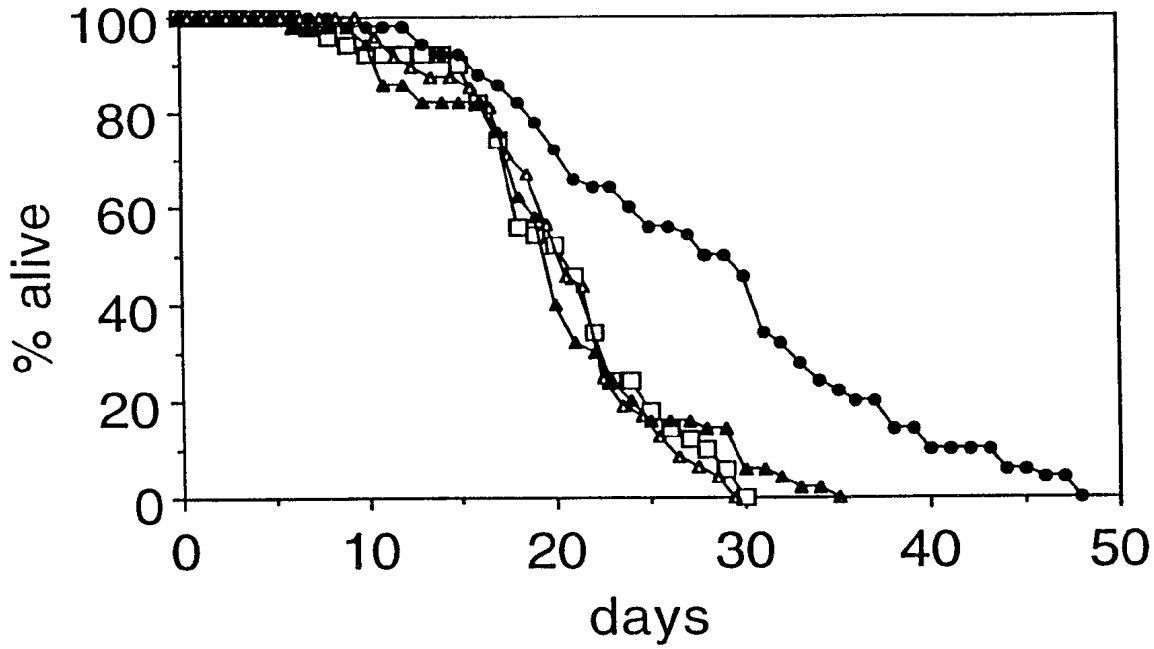

FIG. 2

```
Cat5p/Coq7p  MFPYFYRREFYSCENVVIFSSKPIQGIKISRIRERYIEIMLSRVSVFKPASRGFSVLSSLKITEHTSAKH
                                                  ↓
CLK-1           MFRVITRGAHTAASRQALIEKIIRVDHAGELGADRIYAGQLAVLQG--SSVGSVIKKMWDEEKEHLDT
Cat5p/Coq7p  TEKPEHAPKCQNLSDAQAAFLDRVIRVDQAGELGADYIYAGQYFVLAHRYPHLKPVLKHIWDQEIHHHNT
Rat                MTLDNINRAAVDRIIRVDHAGEYGANRIYAGQMAVLGR--TSVGPVIQKMWDQEKNHLKK
Mouse                                                    KMWDQEKNHLKK
Human                                                    KMWDQEKDHLKK

CLK-1        MERLAAKHNVPHTVFSPVFSVAAYALGVGSALLGKEGAMACTIAVEELIGQHYNDQLKELLAD-------
Cat5p/Coq7p  FNNLQLKRRVRPSLLTPLWKAGAFAMGAGTALISPEAAMACTEAVETVIGGHYNGQLRNLANQFNLERTD
Rat          FNELMVAFRVRPTVLMPLWNVAGF...AGTALLGKEGGMACTVAVEESIAHHYNNQIRMLMEE-------
Mouse        FNELMIAFRVRPTVLMPLWNVAGFALGAGTALLGKEGAMACTVAVEESIANHYNNQIRMLMEE-------
Human        FNELMVMFRVRPTVLMPLWNVLGFALGAGTALLG
                                                  ↓
CLK-1        ----DPETHKELLKILTRLRDEELHHHDTGVEHDGMKAPAYSALKWIIQTGCKGAIAIAEKI
Cat5p/Coq7p  GTKGPSEEIKSLTSTIQQFRDDELEHLDTAIKHDSYMAVPYTVITEGIKTICRVATWSAERI
Rat          ----DAEKYEELLQVIKQFRDEELEHHDTGLEHD....PAYTLLKRLIQAGCSAAIYLSERF
Mouse        ----DPEKYEELLQVIKQFRDEELEHHDTGLDHDAELAPAYALLKRIIQAGCSAAIYLSERF
```

Fig - 3A

```
                                  ϕ   ϕ   ϕ     ϕ   ϕ   ϕ                 ϕ   ϕ
CLK-1     A  18 LIEKIIRVDHAGELGADRIYAGQLAVLQG-SSVGSVIKKMWDEEKEHLDTMERLAAKHNVPHTVFSPVFSVAAYALGVGSALL  99
          B 100 GKEGAMACTIAVEELIGQHYNDQLKELLA•KELLKILTRLRDEELHHHDTGVEHDGMKAPAYSALKWIIQTGCKGAIAIAEKI 187
Cat5p/    A  90 FLDRVIRVDQAGELGADYIYAGQYFVLAH•PHLKPVLKHIWDQEIHHHNTFNNLQLKRRVRPSLLTPLWKAGAFAMGAGTALI 174
Coq7p     B 175 SPEAAMACTEAVETVIGGHYNGQLRNLAN•KSLTSTIQQFRDDELEHLDTAIKHDSYMAVPYTVITEGIKTICRVAIWSAERI 272
Rat       A  10 AVDRIIRVDHAGEYGANRIYAGQMAVLGR-TSVGPVIQKMWDQEKNHLKKFNELMVAFRVRPTVLMPLWNVAGF...AGTALL  90
          B  91 GKEGGMACTVAVEESIAHHYNNQIRMLME•EELLQVIKQFRDEELEHHDTGLEHD....PAYTLLKRLIQAGCSAAIYLSERF 178
Mouse     A                                    KMWDQEKNHLKKFNELMIAFRVRPTVLMPLWNVAGFALGAGTALL
          B     GKEGAMACTVAVEESIANHYNNQIRMLME•EELLQVIKQFRDEELEHHDTGLDHDAELAPAYALLKRIIQAGCSAAIYLSERF
Human     A                                    KMWDQEKDHLKKFNELMVMFRVRPTVLMPLWNVLGFALGAGTALL
```

Fig - 3B

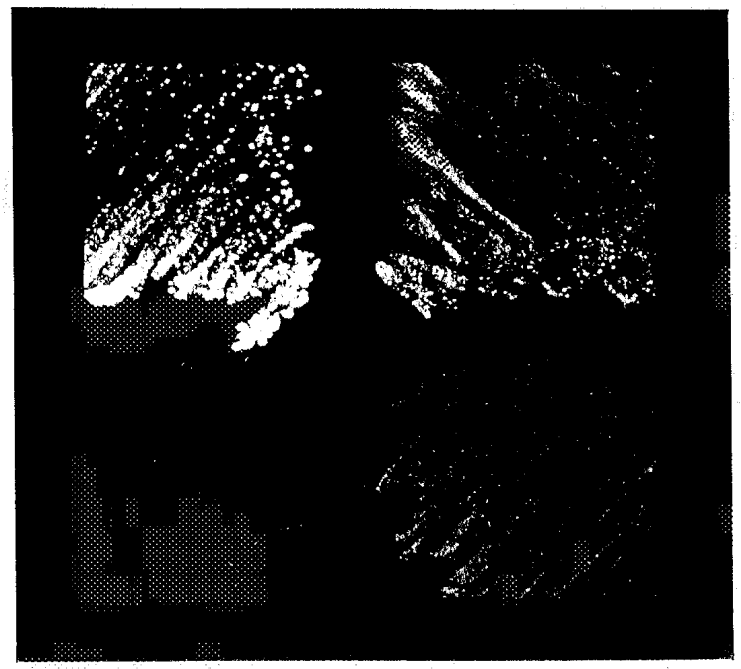

FIG 4

Sequence of TOC-1 and its homologues

```
                                                .     . . . . .    .   .. .      ...    .
H.sapiens   MGTIHLFRKPQRSFFGKLLREFRLVAADRSMGRYMLFGVINLICTGFLLMWCSSTNSIALXAYTYLTIFDLFSLM
C.elegans                   MVSFSWVSRSLHHPQGKRGLISTLICLACVGILAYCVSTSHSIVLMSTLWITIFSFCSQF

              . .   .  ..    . .  .  .....  ......   .  ... .  .
H.sapiens   TCLISYWVTLRKPSPVYSFGFERLEVLAVFASTVLAQLGALFILKESAERXLEQS--------------------
C.elegans   ASLYSMSIT-EKPTHKFSYGLARVPVLAVFSTTVLAQLFSIFLSKESFEHLLSPDHHGSHDASAAHEHEVEEIGG

H.sapiens   ------------------------------------------------------------------------
C.elegans   WPYFVGSAASSVALLLSAYALKNQPFQHVLQSATASSLQEHAADLSHAVCWVIPGLSRLLLPRINSMVLLALTTT

                                                . . . .   ....... . . .  . . .  .....
H.sapiens   ---------------------------XLXLCIPXSVYSGKVXLQTTPPHVIGQLDKLIREVSTLDGVLEVRNEH
M.musculus                                      YPMSVYSGKVLLQTTPPHVIGQLDKLIREVSTLDGVLEVRNEH
C.elegans   GLNLLCEHFKHDFAWADPVCCLLLSVAVFSTMYPLSTYTGMILLQTTPPHLINQIDRCISEASHIDGVLELKSGR

              .. . . .. . . .. .... .. .....           ....  ..  .
H.sapiens   FWTLGFGSLAGSVHVRIRRDANEQMVLAHVTNRLYTLVSTLTVQIFKDD-WI---RPGFT
M.musculus  FWTLGFGSLAGSVHVRIRRDANEQMVLAHVSNRLCTLVSTLTVQIFKDD-WI---RPALSSGPVAPNVLNFSDHH
C.elegans   FWQLDFNSLVGTVDVRVRRDADEQNVLAHVTEKFSSVITVLTVQVVKDAAWSAGEQVPYSNGHIHKSEGNHSHDN

                                    .   .       .                    ..      ..
M.musculus  VIPMPLLKNVDERTPVTSTPAKPS-SPSPEFSFNTPGKNVSPVILLNTQTRPYSLGLNRGHTPYSSVFSQ
C.elegans   GHGHSHDHNDHGHSHGHDDHGHDSHGHSHDHNEHDHGHSHGGNNDNHGHSHSAGSDNHHGHS-HDGVFYH
```

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Fig 5

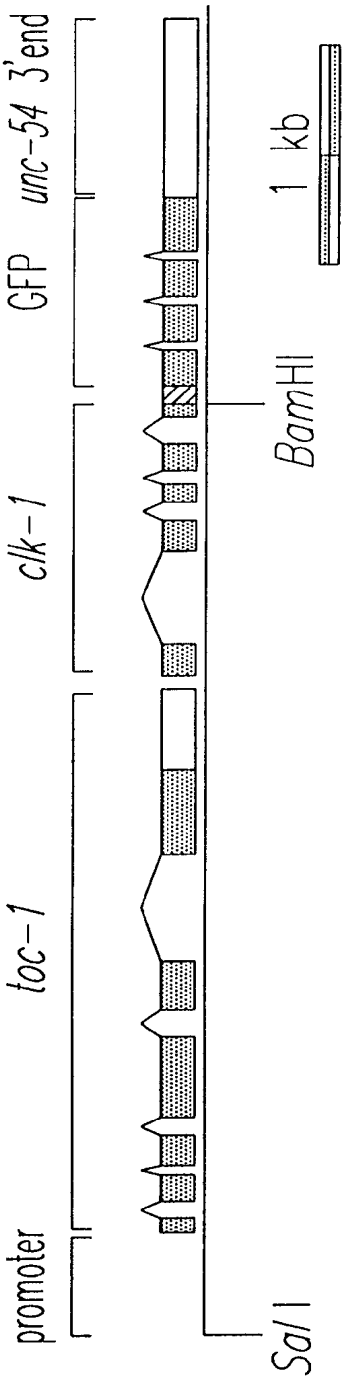

Fig. 6

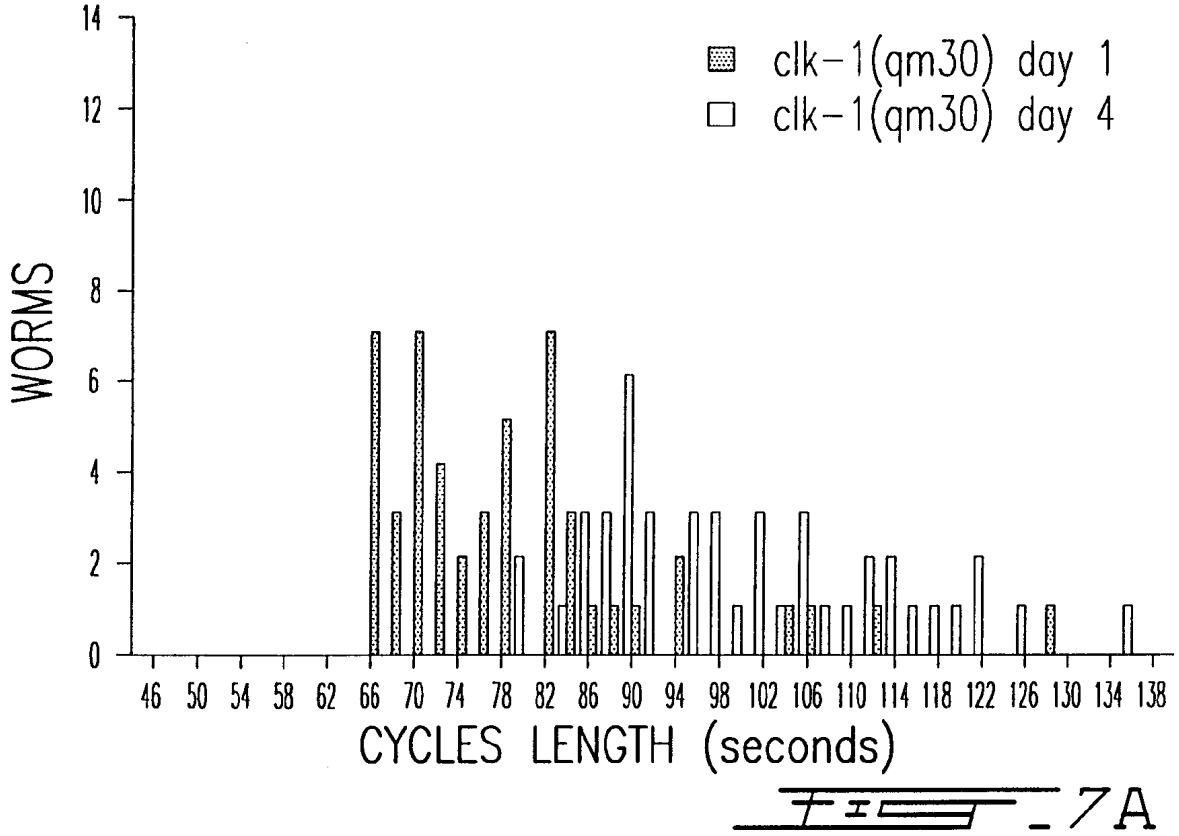
clk-1(qm30) day 1
clk-1(qm30) day 4
WORMS
0
2
4
6
8
10
12
14
46 50 54 58 62 66 70 74 78 82 86 90 94 98 102 106 110 114 118 122 126 130 134 138
CYCLES LENGTH (seconds)

Fig. 7A

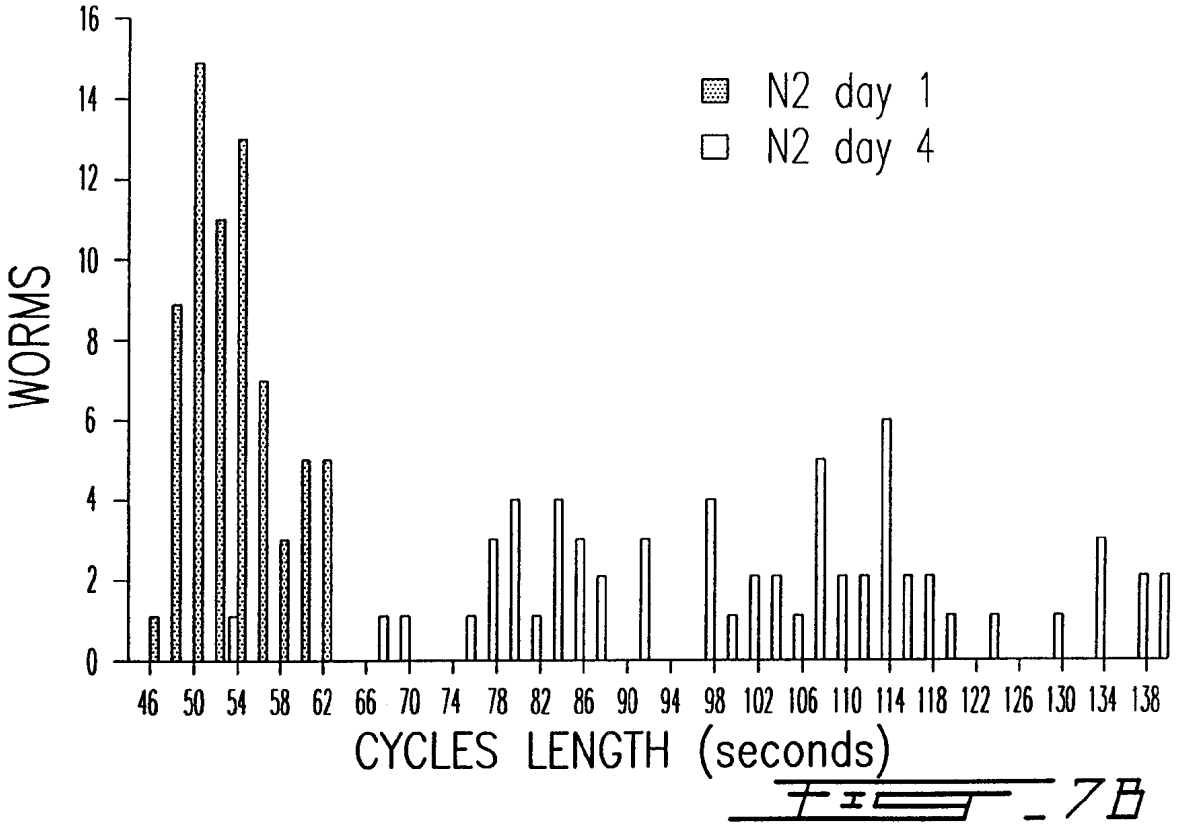
N2 day 1
N2 day 4
WORMS
0
2
4
6
8
10
12
14
16
46 50 54 58 62 66 70 74 78 82 86 90 94 98 102 106 110 114 118 122 126 130 134 138
CYCLES LENGTH (seconds)

Fig. 7B

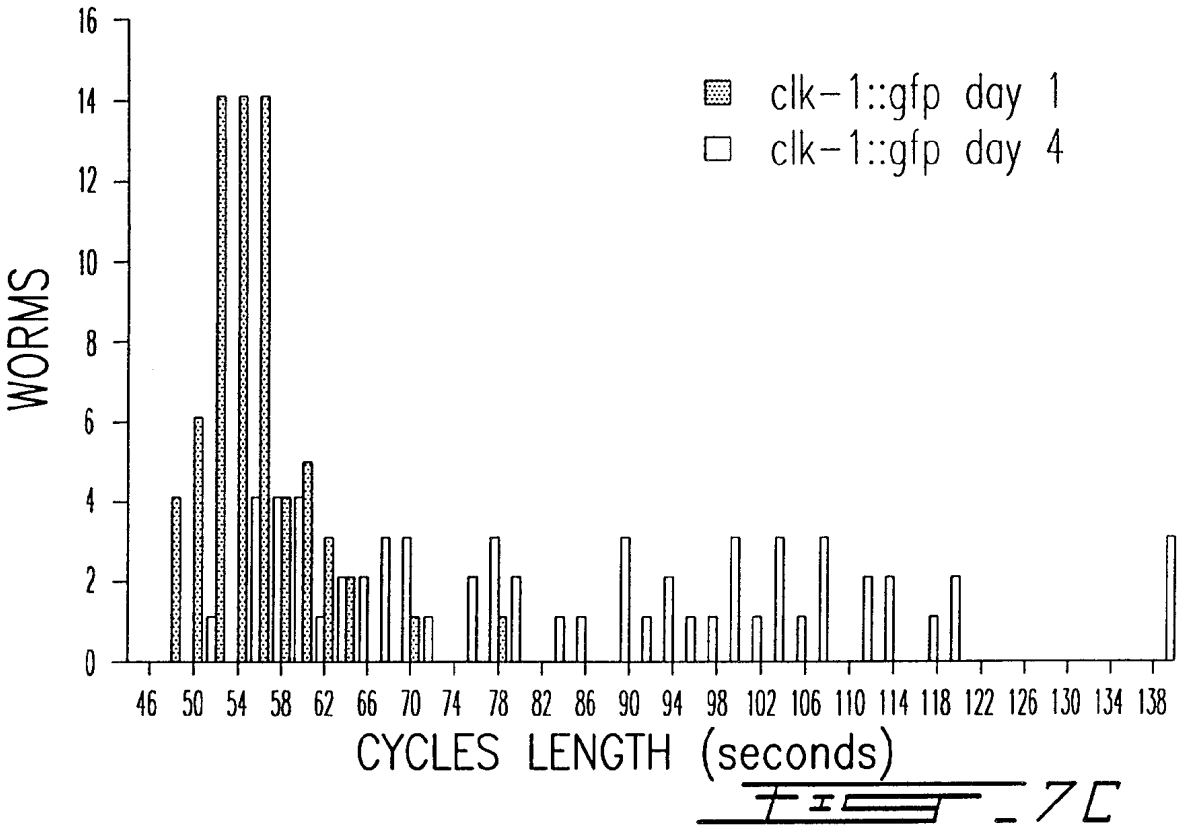
clk-1::gfp day 1
clk-1::gfp day 4
WORMS
0
2
4
6
8
10
12
14
16
46 50 54 58 62 66 70 74 78 82 86 90 94 98 102 106 110 114 118 122 126 130 134 138
CYCLES LENGTH (seconds)

Fig. 7C

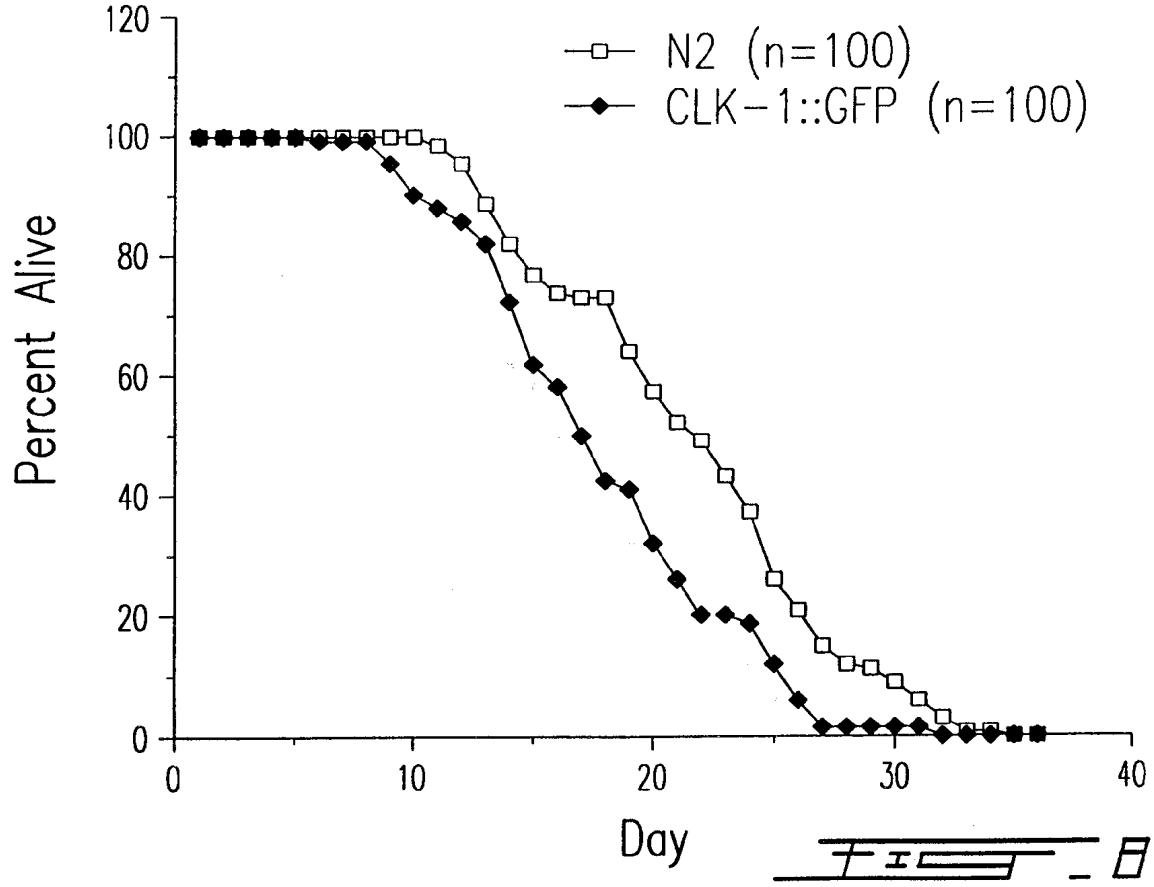
N2 (n=100)
CLK-1::GFP (n=100)
Percent Alive
0
20
40
60
80
100
120
0
10
20
30
40
Day

Fig. 8

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/CA 97/00768

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 A01K67/027 A61K35/00 //C07K14/435

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WONG A ET AL: "Mutations in the CLK-1 gene of Caenorhabditis Elegans affect development and behavioral timing" GENETICS, vol. 139, no. 3, March 1995, pages 1247-59, XP002054192 cited in the application see page 1251, paragraph 4; table 1 ---	1
X	MURAKAMI S ET AL: "A genetic pathway conferring Life Extension and Resistance to UV stress in Caenorhabditis Elegans" GENETICS, vol. 143, no. 3, July 1996, pages 1207-18, XP002054193 see page 1216, paragraph 2 ---	1

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 1998	23.02.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Osborne, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/CA 97/00768

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KENYON C: "Ponce d'elegans: Genetic quest for the fountain of youth" CELL, vol. 84, February 1996, pages 501-4, XP002054194 see page 503, paragraph 8 ---	1
A	JAZWINSKI S: "Longevity, genes, and aging" SCIENCE, vol. 273, July 1996, pages 54-59, XP002054195 cited in the application -----	

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 97/00768

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claim 5
is directed to a method of treatment of the
human/animal body , the search has been carried out and based on the
alleged effects of the compound.